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16. ABSTRACT The ever-rising cost of energy provides incentives for the utilization of waste crankcase oil (WCO) for space heating. The potential health hazards of emissions and waste products resulting from the combustion of WCO are unknown. The toxicity of the emission particles and waste products from two different types of burners, a Dravo atomizing oil burner (AOB) and a Kroll vaporizing oil burner (VOB), are evaluated using automotive WCO. Samples are characterized by performing elemental analysis and scanning electron microscopy. Both burners emitted fine respirable particles. The AOB emission particles contained high concentrations of toxic heavy metals, especially Pb. The VOB retained a significant amount of heavy metals in the burner residue and emitted a much smaller quantity into the air. The toxicity of AOB emission particles, VOB emission particles, and VOB waste residue is evaluated in three bioassay systems, i.e., a rabbit alveolar macrophage cytotoxicity in vitro assay, an intratracheal injection infectivity assay, and a peritoneal irritancy test in mice. The emission particles from both burners and leachate from VOB residue produce a dose-related reduction in viability and cellular adenosine triphosphate (ATP) in macrophages following 20-hr exposure. Mortality due to infection with aerosolized Streptococcus sp. is not significantly elevated by intratracheal injection of AOB emission particles at 100 µg/mouse, whereas a similar dose of VOB ash produced significant elevations in mortality (61.1%). Emission particles from both burners are comparable in overall potency to the most toxic combustion particles tested previously, with the exception of AOB particles in the infectivity assay.

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Toxicity of Particles Emitted from Combustion of Waste Crankcase Oil: *In Vitro* and *in Vivo* Studies

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Toxicity of Particles Emitted from Combustion of Waste Crankcase Oil: *In Vitro* and *in Vivo* Studies. MUMFORD, J. L., HATCH, G. E., HALL, R. E., JACKSON, M. A., MERRILL, R. G., JR., AND LEWTAS, J. (1986). *Fundam. Appl. Toxicol.* 7, 49–57. The ever-rising cost of energy provides incentives for the utilization of low-cost waste crankcase oil (WCO) for space heating. Although WCO is known to contain toxic heavy metals, the potential health hazards of emissions and waste products resulting from the combustion of WCO are unknown. Thus, the toxicity of the emission particles and waste products from two different types of burners, a Dravo atomizing oil burner (AOB) and a Kroll vaporizing oil burner (VOB), is evaluated using automotive WCO. Samples are characterized by performing elemental analysis and scanning electron microscopy. Both burners emitted fine (≤ 3 μm), respirable particles. The AOB emission particles contained high concentrations of toxic heavy metals, especially Pb, which showed concentrations as high as 7.5%. The VOB retained a significant amount of heavy metals in the burner residue and emitted a much smaller quantity into the air. The toxicity of AOB emission particles, VOB emission particles, and VOB waste residue is evaluated in three bioassay systems, including a rabbit alveolar macrophage (RAM) cytotoxicity *in vitro* assay, an intratracheal injection infectivity assay, and a peritoneal irritancy test in mice. The emission particles from both burners and leachate from VOB residue produce a dose-related reduction in viability and cellular adenosine triphosphate (ATP) in alveolar macrophages following 20-hr exposure. Acidity of the RAM medium due to the presence of VOB emission particles and waste leachate contributes to its toxicity. Mortality due to infection with aerosolized *Streptococcus sp.* is not significantly elevated by intratracheal injection of AOB emission particles at 100 μg/mouse, whereas a similar dose of VOB ash produced significant elevations in mortality (61.1%). Emission particles from both burners cause a dose-related sensory irritant response when injected ip in mice. The WCO emission particles from both burners are comparable in overall potency to the most toxic combustion particles tested previously, with the exception of AOB particles in the infectivity assay.

The sharp increase in the cost of energy, in particular heating oil, provides the economic incentives for the utilization of abundant, low-cost, waste crankcase oil (WCO) for heating. WCO is currently used for space heating in large business areas such as garages or gas stations. WCO has been reported to contain toxic and carcinogenic components, including polycyclic aromatic hydrocarbons (PAH) and heavy elements. WCO is mutagenic in the *Salmonella typhimurium* assay (Payne and Martins, 1978), and Grimmer *et al.* (1982) have shown that WCO produced skin tumors in rodents. In studies on the heavy metal content of WCO, the lead content has been reported as high as 45,000 ppm, with an average of 8,400 ppm (American Petroleum Institute, 1975). Also, WCO has been reported to cause poisoning of cattle (Osweiler *et al.*, 1973).

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However, few studies have been reported on the health effects of the emissions from WCO combustion. Hall *et al.* (1983) showed that emissions of nitric oxide and sulfur dioxide from WCO combustion were significantly higher than the emissions from No. 2 fuel oil combustion. Cooke *et al.* (1984) reported that the emissions (semivolatile organics and particles) from WCO combustion contained PAH and were mutagenic in the *S. typhimurium* assay. Nevertheless, little information is available regarding the toxic effects of emission particles and waste products from WCO combustion. Because WCO contains high levels of heavy metals, this study investigated the physical characteristics, chemical composition, and toxicity of emission particles and waste products from combustion of waste oil from automotive crankcases. Separate studies have been performed on the mutagenicity of these emissions. Three bioassays were used to assess the toxicity of the WCO emission particles: (1) rabbit alveolar macrophage assay for cytotoxicity *in vitro*, (2) mouse intratracheal injection assay for the effects on host defense by determining the enhancement of bacterial infectivity, and (3) peritoneal irritation test in mice.

Alveolar macrophages have been widely used to evaluate and to screen the toxicity of inhaled contaminants *in vitro* and *in vivo* (Allison, 1970; Waters *et al.*, 1974; Aranyi *et al.*, 1979). These scavenger cells in the lung play an essential role in defending the host against inhaled particles (Green, 1969). The viability and cellular functions of alveolar macrophages, however, can be adversely altered by exposure to environmental particles. The rabbit alveolar macrophage (RAM) system was used *in vitro* to evaluate the cytotoxicity of the WCO combustion emissions and residue.

Two *in vivo* assays were chosen to assess the potential toxicity of the emission particles from both burners. The mouse intratracheal infectivity assay measures the ability of intratracheally instilled particles to enhance susceptibility to aerosolized, *Streptococcus*-induced pneumonia (Hatch *et al.*, 1981). The mouse peritoneal irritation test provides an estimate of the ability of a particle to produce sensory irritation, and appears to have relevance to respiratory sensory irritation tests (Hatch *et al.*, 1982). These *in vivo* tests were chosen because they require only small amounts of test particles and they have been used previously to test the effects of a variety of particulate matter (Hatch *et al.*, 1985).

METHODS

Sample Collection

The automotive waste crankcase oil used in this study was obtained from a dealer who collects waste oil from the local gasoline stations. Particulate emission samples were collected from two types of waste oil heaters. Both heaters were new and were run within the manufacturer's specifications. One was a Kroll Model W400L waste oil heater, rated at 35.2 kW (120,000 Btu/hr heat input), which uses a pot burner to vaporize the fuel before combustion. This type of burner (a vaporizing oil burner, VOB) emitted a low level of particles from the stack (0.22 mg/m^3) while generating more pot residue (0.02 g/g oil) after combustion. The other type of burner used was a Dravo Hastings Thermoflo Model 20-W0 waste oil heater (an atomizing oil burner, AOB), rated at 73.3 kW (250,000 Btu/hr heat input), in which the fuel is air-atomized and then combusted. It emitted a higher particulate level (20.9 mg/m^3) in the stack than VOB but generated very little pot residue. A dilution tunnel which simulated the dilution and mixing that would occur in the flue gas were emitted directly into the ambient environment was employed for the collection of particles from the two burners. A slip stream of the flue gas was ducted to the dilution tunnel, where the discharge was mixed with filtered air at the ratio of 12 to 1. The emission particles were collected by a massive air volume sampler (Mitchell *et al.*, 1978; Jungers *et al.*, 1981), which was connected to the dilution tunnel. The massive air volume sampler (fabricated by Battelle-Columbus Laboratories, Ohio) was designed to collect large quantities of particulate aerosols in three size ranges, using two impactor stages followed by a high-efficiency electrostatic precipitator (55 steel plates coated with conductive Teflon). The flow rate is 160 ft^3/min (as compared to a nominal 40 ft^3/min high-volume sampler flow), and only the electrostatic precipitator was used during the sampling in this study.

Particle Characterization

The physical characteristics, including morphology and particle size, of the emission particles from the two burners were examined by using scanning electron microscopy

(SEM). The particles of the ground VOB residue were also examined to estimate particle size. X-ray fluorescence analysis was performed on these samples to determine the major inorganic elements present. Samples of the emission particles from both burners and the VOB residue were also analyzed for elemental content by atomic absorption spectrometry (American Society for Testing and Materials, 1978).

In Vitro Cytotoxicity Assay

The samples of the waste oil, the emission particles from both types of burners, and the VOB residue were assayed in rabbit alveolar macrophage cultures to evaluate the cytotoxicity. Prior to assaying, the VOB residue was ground to small particles by a mortar and a pestle and then by a ball mill. Leachate of the VOB residue was also bioassayed and was prepared by mixing 87 g of the residue with 500 ml of deionized water for 48 hr and then filtering the mixture. The alveolar macrophages were obtained from New Zealand rabbits (Charles River Breeding Laboratory, Wilmington, Mass.) by tracheobronchial lavage according to the technique of Myrvik *et al.* (1961). Cell preparations and toxicity assays were conducted as previously described (Garrett *et al.*, 1981). Briefly, cells were plated at 10^6 cells/ml in Costar 6-well cluster dishes in medium M-199 (Hanks') supplemented with 10% heat-inactivated fetal calf serum and antibiotics. The particulate samples were added to a final concentration of 1000 μg/ml after being preincubated 20 hr at 37°C. Cultures were then incubated 20 hr at 37°C in a humidified, 5% CO_2 in air atmosphere on a rocking platform. Samples producing a marked change in medium pH were evaluated with and without the pH adjusted to neutral. After incubation the cells were harvested by trypsinization and aliquots were taken for determining cell number, viability, viability index, and adenosine triphosphate (ATP) levels. Cell number was determined by enumeration using a hemacytometer, viability by trypan blue exclusion, and ATP was assayed by luminescence using the luciferin–luciferase assay. Viability index was determined by multiplying viability (%) by a ratio of experimental cell number to control cell number. Each experimental point was evaluated in triplicate in duplicate experiments, giving a total of six replicates.

The samples which produced a 50% or greater reduction in at least one of the toxicity parameters were further evaluated in dose–response experiments. Particulate samples were tested at 10, 50, 100, 250, 750, and 1000 μg/ml. The dose–response data were used to estimate 50% effective concentration (EC50), which was defined as the sample concentration required to reduce cell functions to 50% of the control values. The data were mathematically transformed, and then a straight line was fitted to the data points by the method of least squares. Concentrations were expressed as the natural logarithm of the dose in micrograms per milliliter. Cell viability was normally transformed to the arc sine of the square root of the fraction of viable cells. A straight line was fitted to the ATP data (in percentage of control) with the logarithm of the dose. The EC50 values were obtained from the simple regression line according to the method of Garrett and Stack (1980).

Mouse Intratracheal Infectivity Assay

Techniques for assessing the ability of particles to increase susceptibility to bacterial pneumonia were the same as those used in previous studies of the effects of metals and fly ash particles (Hatch *et al.*, 1981, 1985). Suspensions of particles in unbuffered saline were intratracheally injected into mice prior to exposure of the mice to aerosolized group C *Streptococcus sp.* A dose of particles equivalent to 100 μg/mouse in 10 μl volume was chosen because it was the lowest dose causing a wide range of responses with the different particles in the previous studies. Also, no measureable toxic effects occurred at this dose due to injection of particles alone. The severity of the ensuing infection was quantified by recording the mortality of the mice over a 15-day period. Percent mortality caused by infection was calculated for at least three treatment groups, each consisting of 30 mice. Excess mortality resulting from particle injections was derived by subtracting the percent mortality in saline-injected control animals from that seen in groups injected with particles.

Mouse Peritoneal Irritancy Test

Procedures used for this test have been described previously (Hatch *et al.*, 1982). Briefly, mice were restrained in a Plexiglas holder, held by the tail, and injected intraperitoneally with 0.2 ml of solution in the midventral surface of the abdomen. After injection, the mice were placed in a box with opaque walls and observed for up to 3 min. Mice showing an irritant response were euthanized immediately by cervical dislocation, and all others were euthanized after 3 min. A positive irritant response was defined as contraction of abdominal and back musculature such that indentations were visible on the dorsum of the mouse. Six concentrations of combustion particles, ranging from 0.1 to 1.0% w/v in saline were injected and data recorded according to the up-and-down format of Dixon and Massey (1957). When data from at least 50 mice had been accumulated, the 50% effective dose (ED50) and confidence limits were determined by probit analysis using the POLO program of Russell *et al.* (1977).

RESULTS

Physical and Chemical Characterization

The physical appearance of the emission particles from the AOB and the VOB is very different. AOB emission particles are fine, powdery, and tan; whereas the VOB emission

particles appear flaky and black, resembling soot particles, and tend to agglomerate. Figures 1 and 2 are SEM photos of the emission particles. Figure 1 shows that the AOB particles, mostly ≤ 1 μm, are of two types, the fabric-like soot particles and the spheric inorganic particles. X-ray analysis indicated that the spheric particles contained Mg, Al, Si, P, Pb, Ca, Fe, and Zn. VOB emission particles were mostly ≤ 3 μm. Figure 2 shows two types of VOB particles; the "smooth" particle on the left contained mainly S and Zn, whereas the irregular particles on the right mainly contained Al, Si, and Pb. A SEM photo of ground AOB residue (not shown) indicated that most particles are larger than 10 μm.

Elemental Analysis

Table 1 shows the results of elemental analysis by atomic absorption spectrometry. The WCO contained significant amounts of Ca, Fe, Pb, Zn, and a high P concentration. All of these elements were also found in high concentrations in the emission particles and waste products of waste oil combustion. The elemental content of AOB emission particles and VOB residue is similar. Both contained highly elevated concentrations of P, Zn, and Pb (7.5% Pb for AOB emission particles and 8% Pb for VOB residue). High concentrations of Mg, Cr, Cu, Mn, and Sb were also found in these two samples. The VOB emission particles showed very high P content (47.6%) and lower concentrations of the metals.

Rabbit Alveolar Macrophage Assay

Results of the RAM assay are summarized as EC50 values in Table 2. Little toxicity was found in the WCO samples; however, the

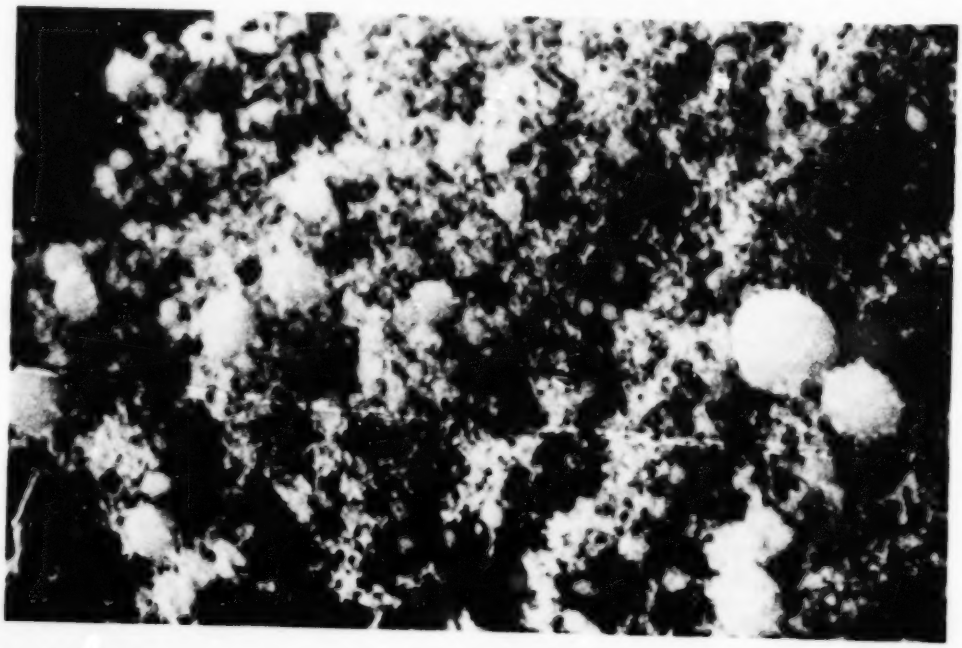

FIG. 1. Scanning electron micrograph of emission particles from atomizing oil burner, using automotive waste crankcase oil, ×9000.

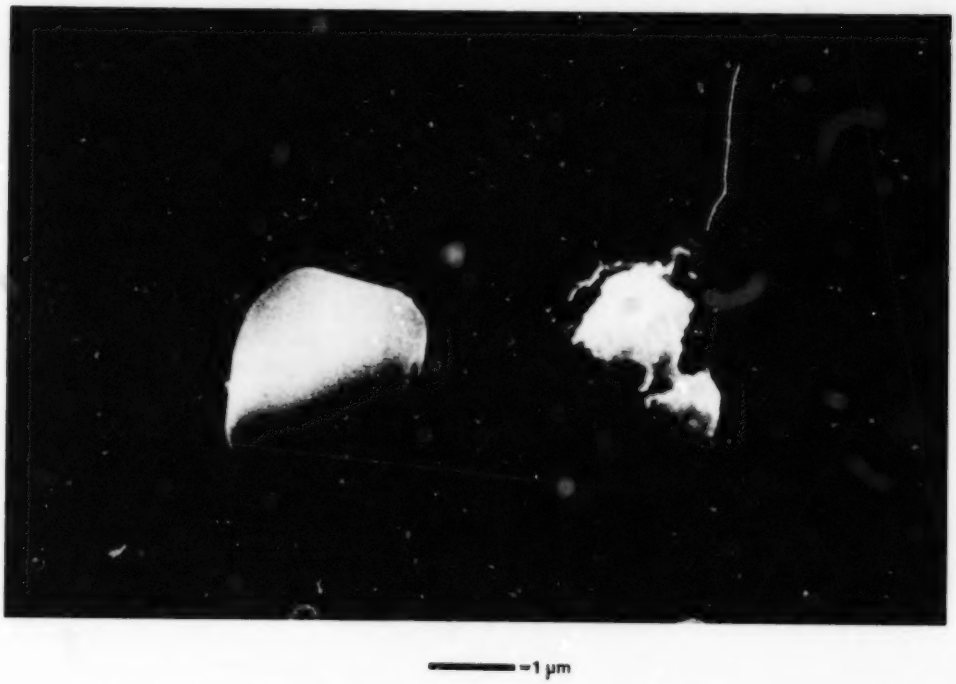

FIG. 2. Scanning electron micrograph of emission particles from vaporizing oil burner, using automotive waste crankcase oil, ×10,000.

emission particles and residue from the WCO combustion showed dose–response cytotoxicity. The VOB samples indicated low pH (2–4) and were evaluated with and without a pH adjustment. For all the samples, cellular ATP was more affected than the viability. ATP data showed that the AOB emission particles were the most toxic, followed by VOB emission particles (pH unadjusted), and the leachate of VOB residue (pH unadjusted). The particulate samples of VOB residue showed low toxicity. This may be due to the large particle size (≥10 μm) of this residue. Adjustment of pH to neutrality significantly reduced the effects on viability, but not on ATP, of the VOB emission particles. Adjustment of pH in the culture containing the leachate sample of VOB residue eliminated all toxicity, indicating that the acidity of the leachate contributed substantially to the toxic effects.

Intratracheal Infectivity Assay

Three experiments were performed, each examining a saline-injected group, as well as groups injected with 100 μg/mouse of AOB or of VOB particles. Results are summarized in Table 3. Experiment 1 showed an unusually large mortality in saline-injected controls, but other groups appeared fairly consistent. None of the groups injected with AOB particles showed high mortality; therefore, no significant effect on bacterial infectivity could be ascribed to these particles. The VOB particles produced high levels of mortality due to infection in all three groups, yielding overall a significant enhancement of 61% over control mortality.

Mouse Peritoneal Irritancy Test

A total of 54 mice were evaluated following injection of particles from the atomizing

TABLE 1

ELEMENTAL ANALYSIS OF AUTOMOTIVE WASTE CRANKCASE OIL AND COMBUSTION PRODUCTS[a]

Sample I.D.	Antimony	Cadmium	Calcium	Chromium	Cobalt	Copper	Iron	Lead	Magnesium	Manganese	Mercury	Nickel	Phosphorus	Zinc
Waste crankcase oil	25.0	0.1	347.5	BLD[b]	0.25	27.5	130.0	1,075.0	115.0	7.5	BLD	8.5	32,277	265.0
AOB emission particles	380.0	80.5	6,000	120	21.0	800.0	5300	75,600	3700	340.0	BLD	50.0	109,350	23,000
VOB emission particles	270.0	68.0	100.0	BLD	BLD	20.0	220.0	2,800.0	70.0	7.0	12.5	0.1	476,330	415
VOB residue	350.0	56.0	21,200	161.0	17.0	1100	9400	80,000	5000	470.0	<0.02	55.5	14,000	31,000

[a] Analysis by atomic absorption spectrometry. Results are given in micrograms per gram of material.
[b] BLD, below limit of detection: limit of detection for chromium is 0.08 mg/liter, for cobalt, 0.12 mg/liter and for mercury 1 mg/liter.

burner and 74 mice after injection of particles from the vaporizing burner. Figure 3 shows the percentage of mice responding positively to the particle samples at different concentrations injected. Both samples produced a typical dose-related increase in irritancy as higher concentrations of ash were injected. Statistical analysis indicated that the atomizing burner ash was slightly more irritating. ED50 values (and their 95% confidence limits) were 0.36 mg/mouse (0.28–0.45 mg/mouse) for AOB emission particles and 0.59 mg/mouse (0.47–0.73 mg/mouse) for VOB emission particles.

DISCUSSION

The toxicity of emission particles and waste products of automotive WCO combustion from two types of burners was assessed. Because particle size and chemical composition affect the toxicity of particulate samples, efforts were also made to investigate the elemental composition and physical characteristics of these combustion products. Combustion concentrates many heavy metals contained in the waste oil, which are emitted into the air or remain in the waste residue. This study showed that the AOB emits a much larger quantity (95 times) of fine (≤ 1 μm), respirable particles into the air than the VOB. These fine emission particles contain high concentrations of the toxic metals Sb, Cr, Cu, Pb, Mg, Mn, Ni, and Zn; the Pb concentration is particularly high, 7.5%. The VOB retained a significant amount of the heavy metals in the burner residue and emitted a much smaller quantity of fine particles into the air. The VOB residue also contained high concentrations of toxic heavy metals similar to the ones found in the AOB emission particles, including 8% Pb. The VOB emission particles contained lower concentrations of heavy metals. The high P concentrations in the waste oil and its combustion emission and waste products, especially in the VOB emission particles, may be attributed to the use of phosphorus-containing detergent additives in motor oil (Codd *et al.*, 1972). The high concentrations of lead in WCO and its

TABLE 2

EFFECTS OF AUTOMOTIVE WCO AND COMBUSTION PRODUCTS IN THE RABBIT ALVEOLAR MACROPHAGE CYTOTOXICITY ASSAY *in Vitro*

Sample	EC50 (μg/ml)	
	Viability index	ATP
Waste crankcase oil	>1000	>1000
AOB		
Emission particles	355 (290–445)[a]	23 (0–43)
VOB		
Emission particles	330 (248–461)	92 (80–104)
Emission particles, pH adjusted	>1000	109 (84–121)
Residue, ground particles	>1000	415 (363–468)
Leachate	120 (102–153)[b]	101 (89–114)
Leachate, pH adjusted	>1000	>1000

[a] The numbers in parentheses represent the 95% confidence interval; data were obtained from duplicate experiments, providing six replicates for each dose.

[b] The leachate was tested in liquid form, and the unit of the EC50 is in microliters per milliliter.

combustion products may come from a gasoline additive, tetraethyl lead.

In comparison to other environmental particles tested previously, the AOB and the VOB emission particles were each ranked to be among the most toxic according to percent reduction of cellular ATP at the concentration 1000 μg/ml in the RAM assay (Hatch *et al.*, 1985). Twenty-three other environmental particles have been tested previously in the RAM assay, including coal fly ash samples from conventional coal-fired power plants and

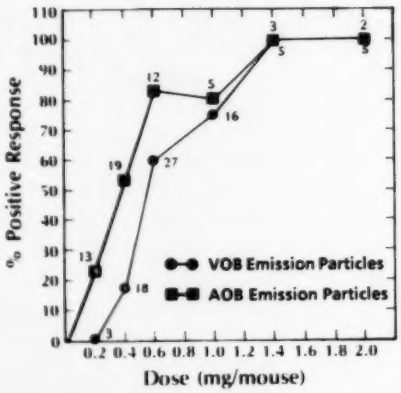

FIG. 3. Effects of emission particles from automotive waste crankcase oil combustion in mouse peritoneal irritation test. The number of mice tested at each dose is given.

TABLE 3

EFFECTS OF AUTOMOTIVE WCO COMBUSTION EMISSION PARTICLES IN INTRATRACHEAL INJECTION[a] INFECTIVITY ASSAY

Sample	Experiment	Dead/total	Average excess mortality (%)
Control (saline)	1	11/30	—
	2	2/30	—
	3	3/30	—
		Mean = 5/30	0
AOB emission particles	1	1/30	—
	2	6/30	—
	3	0/30	—
		Mean = 2/30	−10/0 ± 13.4
VOB emission particles	1	24/30	—
	2	25/30	—
	3	22/30	—
		Mean = 24/30	61.1 ± 9.7[b]

[a] Dose treated at 100 μg/mouse.

[b] Significantly elevated ($p < 0.01$) compared to saline control.

fluidized bed combustion, diesel-exhaust emission particles, volcanic ash, and ambient air particles. Only the highly toxic residual oil fly ash, a German air particulate sample, and silica were comparable in cytotoxicity to these WCO emission particles. For the mouse bacterial infectivity assay, VOB emission particles would also be classed with the most toxic of the other environmental particles studied previously. Other particles which caused a greater than 50% increase in infectivity in the study include residual oil fly ash, coal fly ash from fluidized bed combustion, and a sample of German ambient air particles (Hatch *et al.*, 1985). The AOB emission particles would be classed among the least active of the particles studied previously. It is not presently known why some particles which are known to be toxic in other systems, for example, silica and PbO, reveal no toxicity in the infectivity assay. A possible explanation is that some particles, in addition to being cytotoxic, cause stimulation of phagocyte influx and activity, which counteracts the negative effect of the particles. In the mouse peritoneal irritancy test, AOB and VOB emission particles were ranked as second and fourth most irritating samples among the seven environmental particles tested. These particulate samples included residual oil fly ash, coal fly ash from conventional and fluidized-bed combustion, and volcanic ash. The residual oil fly ash was the most irritating in this assay. Previous studies have shown that the differences in particle irritancy may depend on the presence of irritating metal salts or oxides. Soluble salts of metals such as Cd, Ni, Ca, and V appear to be highly irritating, and some oxides (CdO, CaO) exhibit this property. Acidity or basicity of the particle appears to contribute only slightly to particle irritancy.

The findings of the high toxicity of the AOB emission particles are in agreement with the studies reported by Beck *et al.* (1984). The same AOB emission particles were bioassayed for pulmonary toxicity in the hamster via intratracheal instillation. Extensive pulmonary injury, including increased levels of albumin and extracellular lactate dehydrogenase in bronchoalveolar fluid and impairment of macrophage phagocytosis, was found after 1 day of exposure. The pulmonary toxicity of AOB particles was greater than the highly toxic α-quartz.

This study indicated that the emission particles and waste products from automotive waste crankcase oil combustion, which contain high levels of heavy metals, particularly of Pb, are highly toxic. Further investigations on the health effects of these combustion products are needed.

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03S)ADDITIONAL SRC CLIENTS:
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03C)COMP. ENTRY CODE:
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35)CORPORATE AUTHOR CODE: 007560038
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@10G)C. /Lintz ;D. /Adams ;J. L. /Anderson ;R. F. /Carrillo ;D. M. /Gardner
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(9D) GRAPHICS

(9A) S/A 1 FOR 1 PGT 1 PGS 1 T

(9E) SUBJECT CATEGORIES

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(9B) DESCRIPTORS

* Archaeology,

Site surveys,
Investigations,
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(9I) IDENTIFIERS

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(7A) ABSTRACT PAGES

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